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ULTRASTRUCTURAL INVESTIGATIONS ON HUMAN CAROTID BODIES

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The carotid bodies are compact formations located in the carotid bifurcation. They can be found in the organism of all vertebrates except fish. Their histological patterns are by and large identical, their physiological role is, however, debated. To-day we consider them as chemoreceptors for perceiving the oxygen and carbon dioxid content of blood.

We have studied the structure of carotid bodies for more than 25 years, performing our investigations on human and animal material (hedgehog, dog, horse, pig, cattle, sheep). We used earlier stained and impregnated preparations, and light microscope for the investigations. We discuss below our findings on human material with electron microscope.

Materials and Methods

For the investigations we used human carotid bodies removed in an operative way owing to serious asthma bronchiale. The material was fixed immediately after the operation in 1 p. c. osmium tetraoxid bufferred according to Sorenson, dehydrated in a gradually increasing alcohol series and embedded in araldites. The sections and photographs were prepared partly in London, in the electron microscopic laboratory of the Middle Sex Hospital, Medical School, partly in Budapest in the Central Medical Research Institute, a third part in the Anatomical Institute in Budapest, and the forth part in Szeged, in the Institute of Zoology and Biology of the University.

From the structural elements found in the photographs we deal below with the glomic cells among them with chemoreceptor cells, capsel cells, and then we discuss our observations concerning blood vessels, nerve fibres and synapses.

Glomic cells

The glomerules that form the major part of the substance of carotid bodies and are delimited from one another with septa of connective tissue, consist in most part of glomic cells, blood vessels and nerve fibres.

Among glomic cells we could distinguish two types of cells. One of them is the chemoreceptor cell, the other is the capsel cell. The former ones are comparatively large, roundish cells having no or but a few processes which are rounded off at the end. The capsel cells are prolongated, bearing many processes that are long and surround the bodies resp. processes of the chemoreceptor cells like a cover.

Chemoreceptor cells

The cell membrane is delimited sharply, the cytoplasm is granulated, with a lot of granules and vesicles of various sizes. Among the granules there are major roundish, sometimes ovoid ones. These are the osmiophil bodies to be discussed later. There are much rarer the similarly roundish, large-sized lipoid bodies, covered by strongly osmiophile caps. It isn't infrequent if two pale lipoid bodies are connected together by an osmiophile cortex. The vesicles can be observed in the cytoplasm in very large masses, generally of uniform size but the thickness of walls shows considerable differences. Among the vesicles the cell organs take place from the following are to be observed clearly: 1) endoplasmic reticulum, 2) GOLGI complex, 3) mitochondria, 4) osmiophile bodies, 5) lysosomes, 6) cilia, 7) microvilli. Like characteristic cell formations, the desmosomes appearing here and there join those enumerated above (Fig. 1).

1) The endoplasmic reticulum consists of essentially straight parallel canals with proportionately wide lumen that appears mainly in the prolongations of the cells and also here at the basis in a conspicuous form. Sometimes they form a complicated canal system with wide lumen extending to the whole cytoplasm, some parts of them being meanwhile widened vesicle-like.

2) The GOLGI complex consists of a system of long, welldeveloped canals and of vesicles that can be observed in the form of characteristic fields near the nuclear membrane. The greater part of canals is parallel with the nuclear membrane.

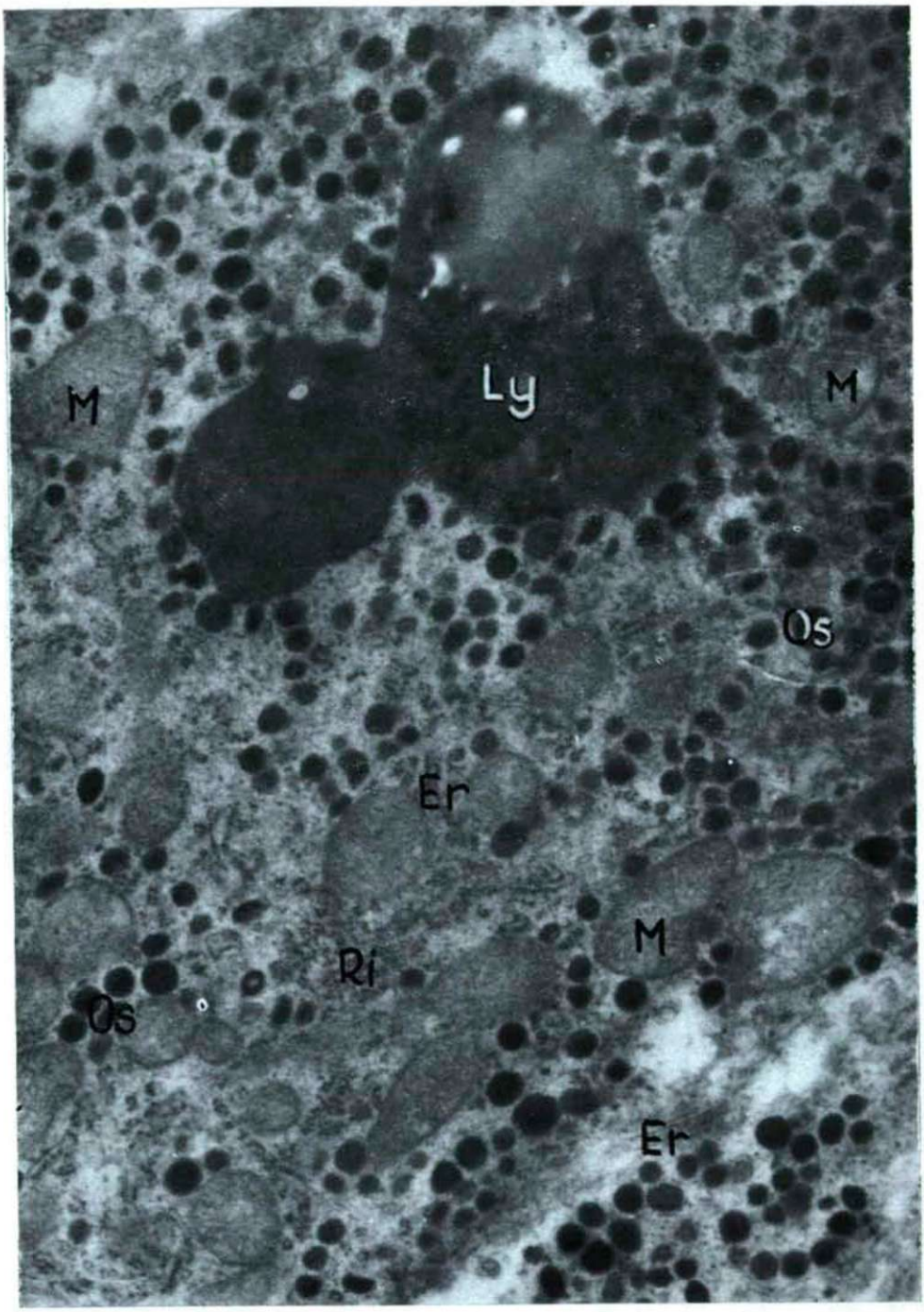
3) The mitochondria are roundish, here and there elongated bodies all of them being constructed according to the crista type. There are frequent the typical dumb-bell-like shapes, their club-like terminal pieces are connected with a thin link. These forms bear reference to mitochondria multiplying by division.

4) The osmiophile bodies or „dense cored" vesicles are characteristic components of the chemoreceptor cells. Essentially they are dark, roundish bodies surrounded by a thin light cortex. According to the investigations in other fields they contain catecholeamin. It is strong phenomenon waiting for interpretation that where there are a lot of osmiophile bodies the mitochondria are few in number and vice versa, where there are a lot of mitochondria there are but a few osmiophile bodies.

5) The lysosomes are polymorphous, sometimes lamelliform dark bodies appear in the chemoreceptor cells here and there in large numbers. They can be seen in particularly great quantities and in large fields if the substance gets into the osmium acidic fixing bath later, after having been prefixed in formaline. Their mass appearance is connected with reducing processes in the cells (Fig. 2).

6) Cilia can be found in a comparatively low number but there are places where they are present in great quantities. In material of animal origin cilia had been found by other researchers as well (BISCOE and STEHBENS, 1966; HESS, 1968). These cilia are constructed, according to literary data as to follow type 9 + 0. These cilia shapes are qualified by some researchers as sensory cilia. The cilia observed by us belong to the type 9 + 2 being, therefore, small motorial organs. Their exact place could not be established, so far, their physiological role is unknown (Fig. 3).

Fig. 1. Homo: carotid body. Chc — chemoreceptor cell, Ca — capsel cell, Cyt — cytoplasm, Er — endoplasmic reticulum, G — Golgi complex, M — mitochondrium, V — vesicles, N — nucleus, Nm — nuclear membrane, Pro — cell process, Sy — synapsis. x 25 000.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7) The microvilli are longer or shorter bulges of the surface. Their shapes are various, their thickness is even. The basis is sometimes wide, containing vesicles of various shapes and sizes.

The desmosomes are the „electron dense" parts on the cell membranes touching each other that considerably differ from one an other both in their length and in depth. They are similar to the synaptic membranes and the intercalated discs.

The nuclei of chemoreceptor cells are roundish and considerably large. The „indentations" of nuclear membrane are frequent, protruding here and there deep into the nuclear substance. The nuclear membrane consists of two lamellae separated from each other by a considerable space. The external lamellae often protrudes deep into the cytoplasm forming and extensive hernia (Fig. 4).

The nuclear pores are wide, round them systems of small granula take place that remind us of the closing structure of the excretory system of *Ciliata*. The granular elliptical systems may play also here a closing role. The nuclear pores, where they appear in larger areas, remind us of plant stomata (Fig. 5).

Capsel cells

The capsel cells are elongated, their nuclei are homogeneous and ellipsoid. The nucleus is surrounded by cytoplasm in the form of a narrow border. The capsel cells can be observed in a larger mass at the edge of glomeruli. They are similar to SCHWANN cells and even there is a possibility that they are simple SCHWANN cells (Fig. 1). This idea arises first of all if we think on the connection of capsel cells with the nerve fibres.

The capsel cells contain, namely, just as SCHWANN cells many neuraxons limited with a mesaxon membrane. The difference between the two cell forms lies in the fact that the SCHWANN cells have double-folded membranes and their connection with the neuraxons is richer than that of the capsel cells (Fig. 6).

Blood vessels

A particular feature of the vascular system is that the adventitia of the minor arteries and veins there are pressoreceptors provided with wide neurofibrillary endplates from which it follows that the carotid bodies participate, apart from chemoreception, in baroreception too.

The greater part of the vessels are sinusoid and capillaries the diameter of which is extremely variable highly dependent upon the prevailing physiological state. In case of the diseases in the area of cardiovascular system, they are dilating strongly. Under normal conditions from the lumen canals protrude between the cells. Their lumens have different diameters. By these canals it becomes possible that when need arises the vessel lumen can be

Fig. 2. Homo: carotid body. Chemoreceptor cell. Er — endoplasmic reticulum, Os — osmiophile body, Ri — ribosomes, Ly — lysosome, M — mitochondrium. x 75 000.

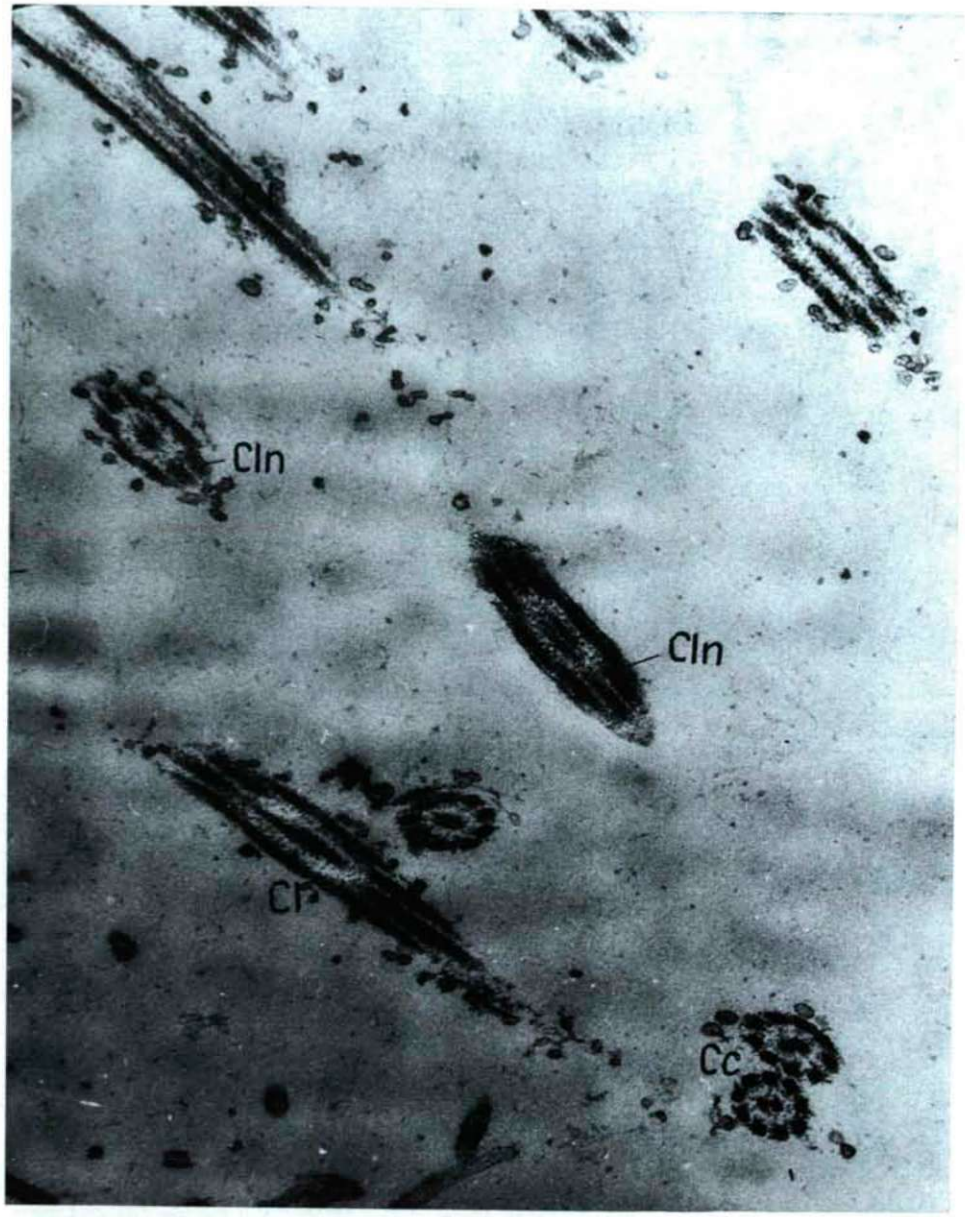

Fig. 3. Homo: carotid body. Cilia. Cc — cilium in cross-section, Cl — cilium in long-section, Cln — column. x 72 000.

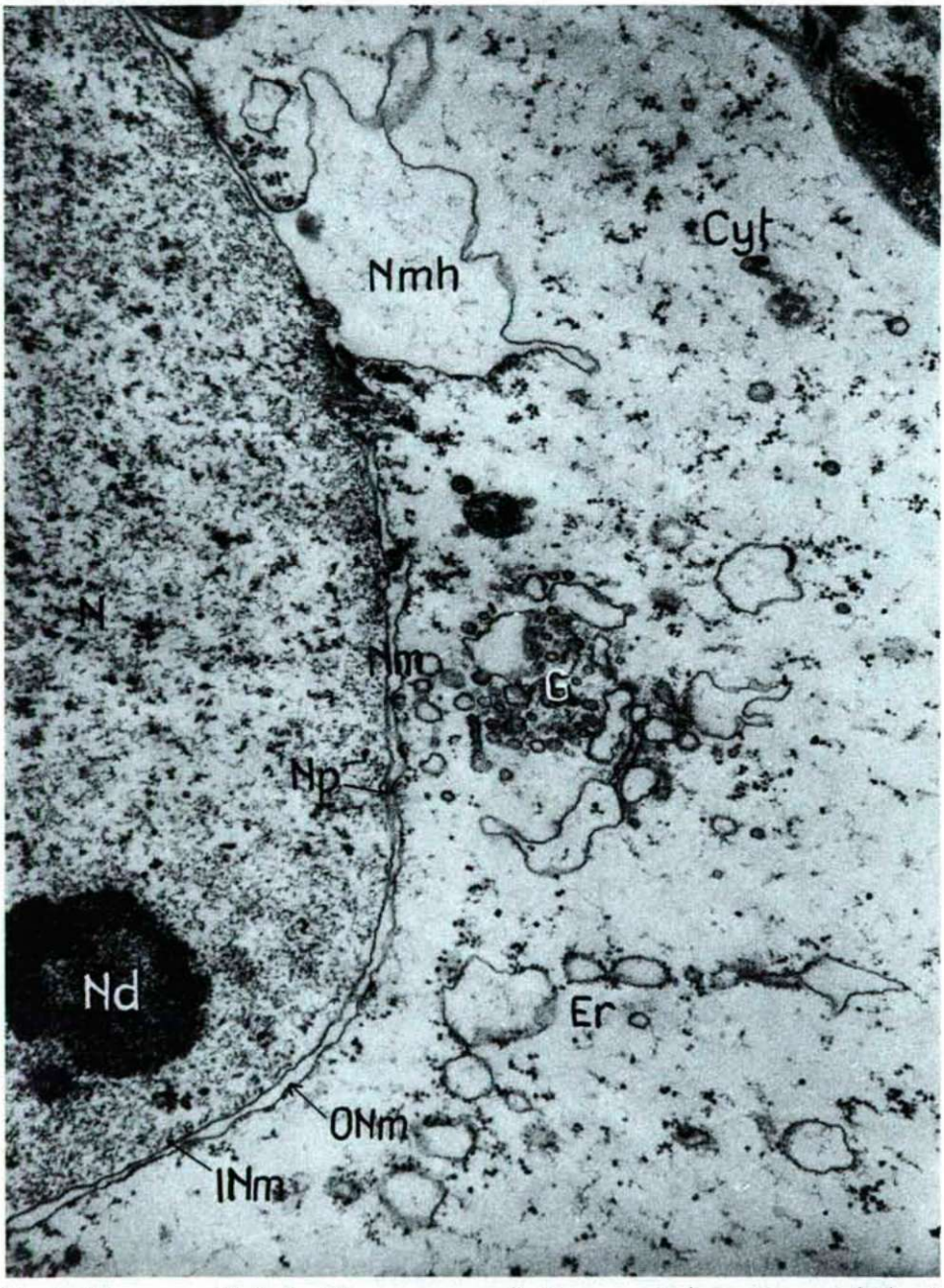

Fig. 4. Homo: carotid body. Chemoreceptor cell. Cyt — cytoplasm, Er — endoplasmic reticulum, G — Golgi complex, N — nucleus, Nd — nucleolus, Nm — nuclear membrane, ONm — outher nuclear membrane, INm — inner nuclear membrane, Nmh — nuclear membrane hernia. x. 56 000.

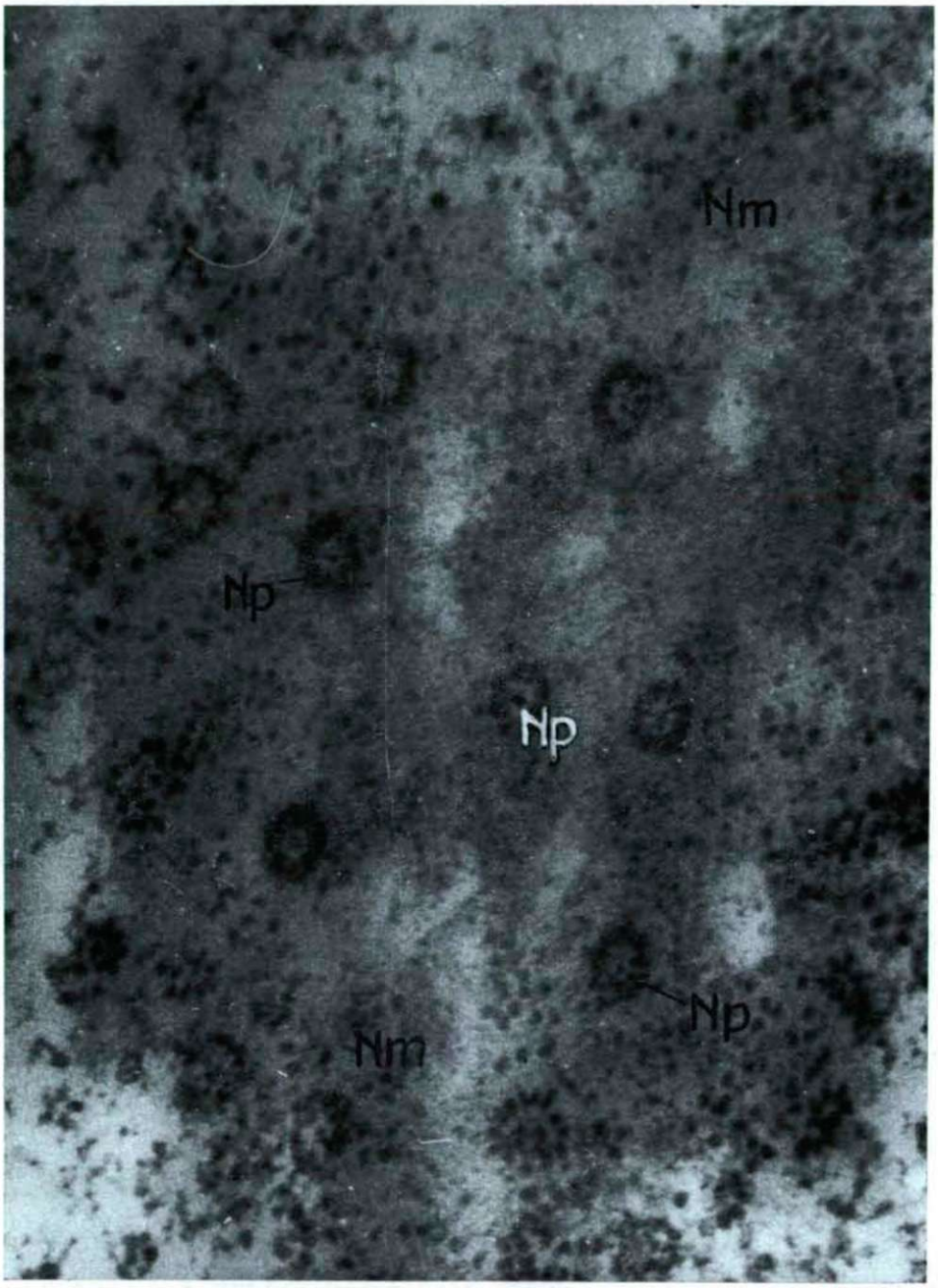

Fig. 5. Homo: carotid body. Chemoreceptor cell. Nuclear pores. Nm — nuclear membrane, Np — pores. x 92 000.

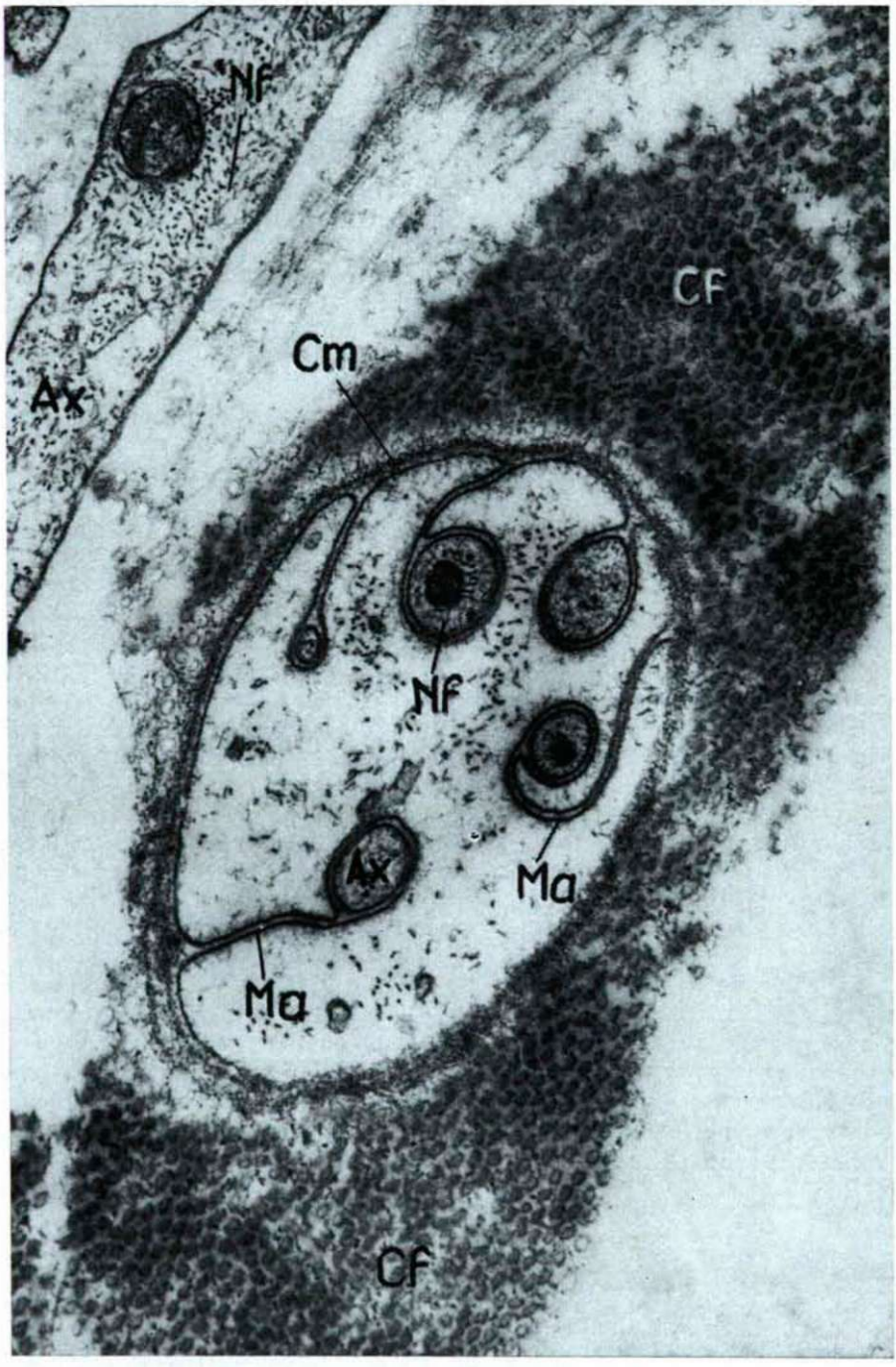

Fig. 6. Homo: carotid body. Schwann cell. Cm — cell membrane, Ax — axon, Ma — mesaxon, Nf — neurofilament, Cf — collagen fibrils. x 36 000.

extremely enlarged. Both the lumens of sinusoid vessels and those of the capillaries are limited by an uninterrupted endothelial cell layer. Then comes the basal membrane that consist of collagenous fibril boundles surrounded by a layer of pericytes (Fig. 7).

The endothelial cells are elongated, their shape is changing and depends upon the saturated state of the lumen. Some of them are brick-like others come up to blunt processes at both ends. The boundary between the cells is sharp. The adjacent cells are connected together with thick desmosomes of wavy course. The cytoplasm is homogenous but smaller or larger granules and vesicles are not seldom contained in them. The characteristic components of the cells are the microvilli at their radical parts with vesicles and here and there with intrusions sitting on a wide basis.

The collagenous fibrils of the basic membrane form a network with large meshes between the endothelium and the pericytes. The latter ones are narrow, elongated cells. Their cell membrane is not sharp, the cytoplasm and nucleus are highly homogenous.

The supposition represented by MARCHAND and later on by F. DE CASTRO, according to which, the wall of capillaries consisting here and there of parenchym cells, is not verified by electron microscopic pictures.

Nervous system

The carotid bodies are supplied abundantly with nerve fibres. The fibres are coming in trunks and bundles of different thickness from the glossopharyngeal nerve, vagus nerve and the ganglion cervicale supremunm. The fibre bundles belonging to different systems form a plexus rich in connective tissue capsule. This is named periglandular plexus. This denomination is attached to F. DE CASTRO's name, dating back to a time when the carotid bodies were thought to have an endocrine function. The plexus coming from the periglandular plexus surrounds the nests consisting of glomus cells. Therefore, it is called peri lomerular plexus. The third plexus is departing from the periglomerular plexus, penetrates inside the glomerule and surrounds the glomus cells with a lense fibre system. The name of this plexus is intraglomerular plexus.

In the sections, impregnated with our recently elaborated method (ÁBRAHÁM, 1968), there appeared sharply not only the various neuroplexuses but also endings were to be observed in large numbers in the form of end rings (Fig. 8). Their number being extremely high, we may draw with reason the conclusion that each of the glomus cells has its own nerve termination and even it is shown by the most pictures obtained, that a glomus cell must be connected with more nerve fibres (ÁBRAHÁM, 1968).

Synapses

All research workers who investigated the carotid bodies with electron microscope (ROSS, 1959; LEVER, LEWIS and BOYD, 1959; DE KOCK and DUNN, 1964; BISCOE and STEHBENS, 1965; GRIMLEY and GLENNER, 1967; HESS, 1968; DE KOCK and DUNN, 1966, 1968; DEARNALEY, FILLENZ and WOODS, 1968;

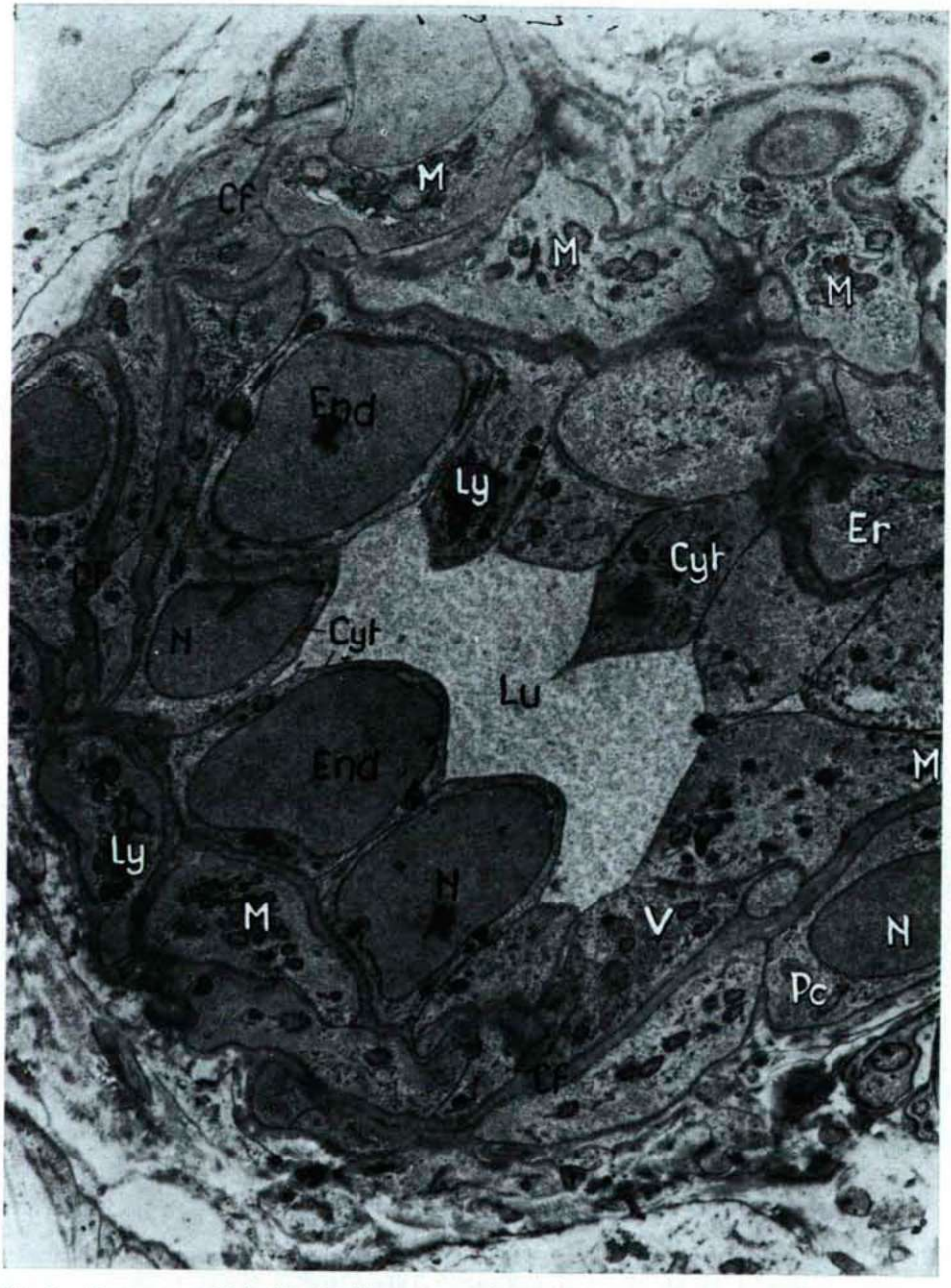

Fig. 7. Homo: carotid body. Capillary. Lu — lumen, End — endothel cell, Pc — pericyte, Cyt — cytoplasm, Er — endoplasmic reticulum, Ly — lysosome, M — mitochondrium, V — vesicles, N — nucleus, Cf — collagen fibrils. x 75 000.

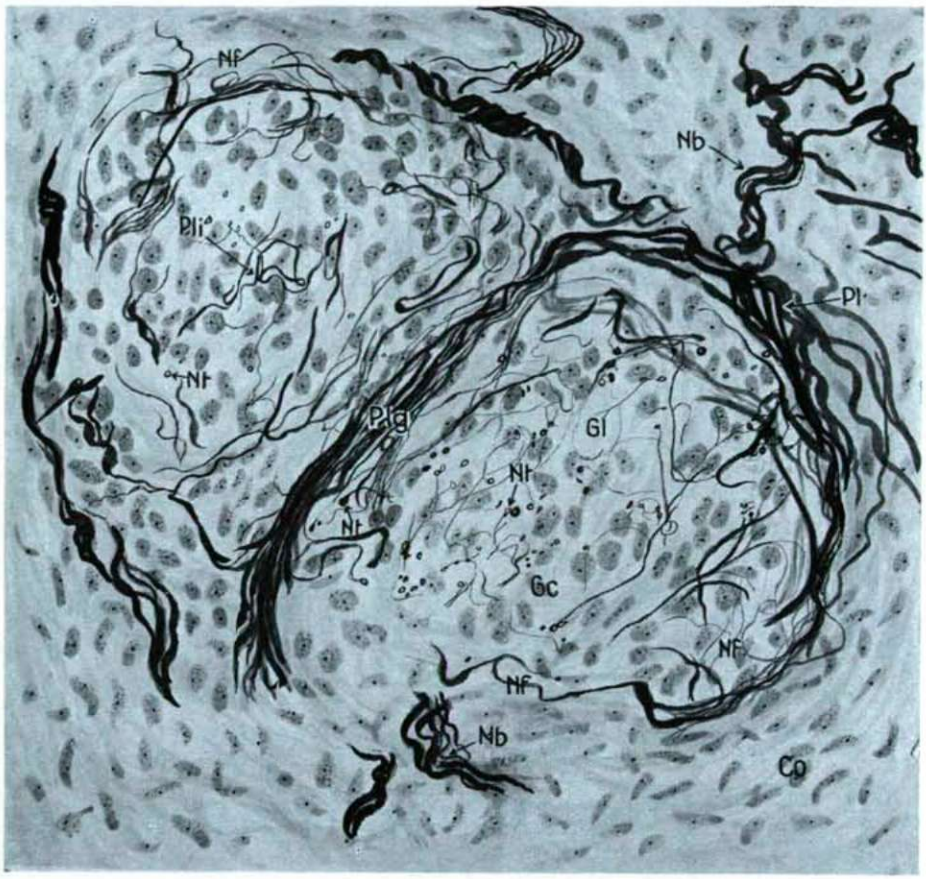

Fig. 8. Homo: carotid body. Nerve supply. Co — connective tissue, Gl — glomerulus, Gc — glomic cell, Nb — nerve bundle, Nf — nerve fibre, Pl — plexus periglandularis, Plg — plexus periglomerularis, Pli — plexus intraglomerularis, Nt — nerve terminal. x 600.

De Castro and Rubio, 1968) have agreed on the fact that there is a synaptic connection of the glomus cells with the nerve fibres that are permeating the glomeruli, in view of the structure of this connection, however, there are great differences between current opinions. We found two forms of the synaptic connections. One of them was observed in a large numbers, the other more somewhat scarcly.

The first form of synapses is a shorter or longer parallel, resp. terminal contact. The situation is in both cases that the neuraxons leaving the Schwann cells the capsel cells respectively are in contact with the bodies of chemoreceptor cells resp. with their processes. In this form of contact, in the axons, the synaptic vesicles, the mitochondria, the neurotubuli and the contact between the axolemma and cell membrane can be seen, in the meeting membranes, however, there are not any synaptic thickenings and in the axons

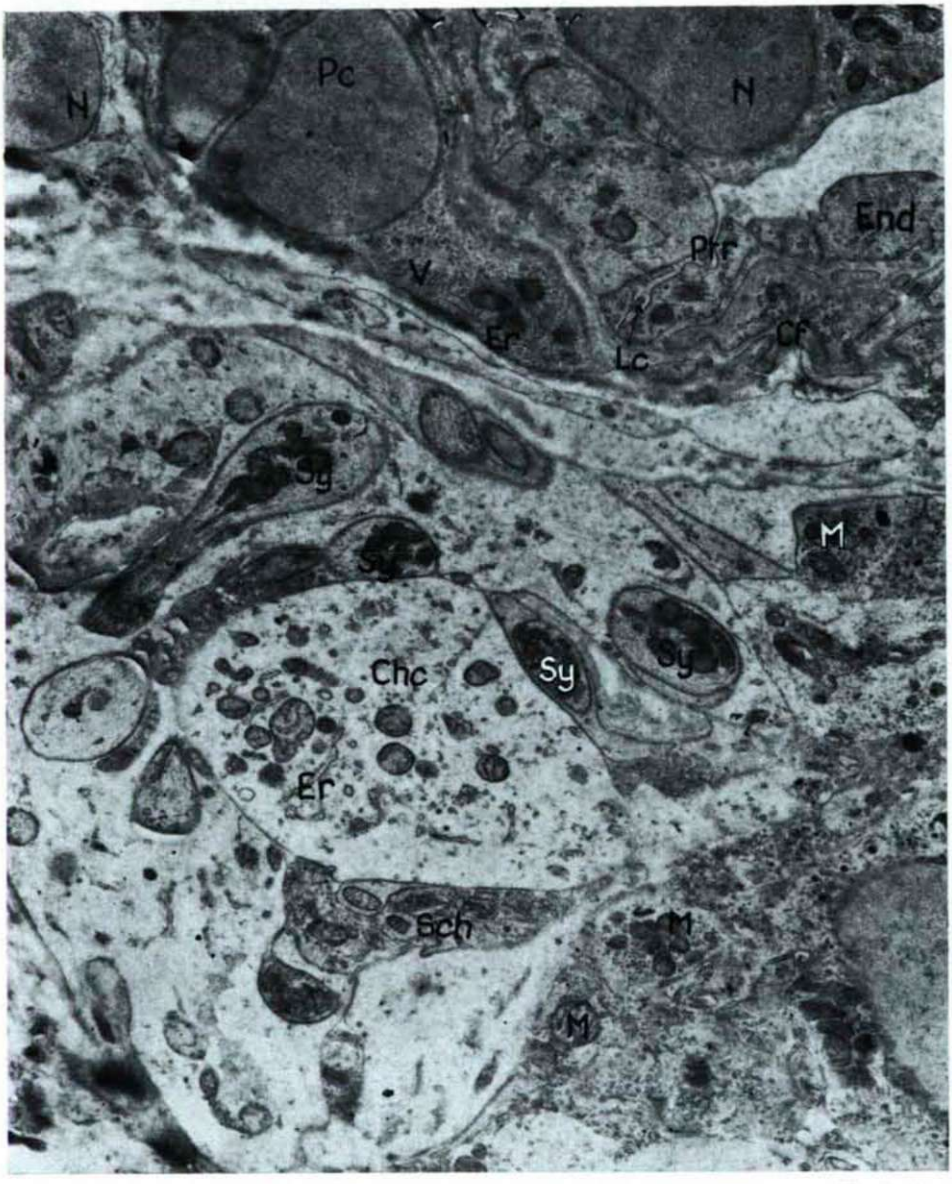

Fig. 9. Homo: carotid body. Afferent synapses. Chc — Chemoreceptor cell, End — endothel cell, Pc — pericyta, Sch — Schwann cell, Er — endoplasmic reticulum, M — mitochondrium, V — vesicles, N — nucleus, Lc — canal, Cf — collagen fibril, Ptr — protrusio, Sy — synapsis. x 25 000.

the grouping consisting of synaptic vesicles are lacking. This synaptic form corresponds to the nerve terminal rings seen through light microscopes. As a consequence of their frequency and pattern, we think that these forms of synapses are afferent synapses conducing impulses from the chemoreceptor cells to the centre in the medulla oblongata (Fig. 9).

The other synaptic form agrees with the forms known from the central nervous system, the striated muscle fibres and the myocardial cells. In these forms we can observe the synaptic vesicles, the synaptic vesicle clusters under the axolemma near to the cell membrane as well the thickenings in the pre- and postsynaptic membrane and the synaptic space (Fig. 10).

As in the synaptic forms of this structure the synaptic vesicle clusters show the direction of impulses although it is impossible to establish with certainly, to where the meeting surfaces belong, we consider as neuraxon that part of the meeting surfaces on which the synaptic vesicles are accumulated. Since in the sensory synapses there are no such clusters, we consider these second forms of the synapses found, as efferent synapses. Among these forms we have found such ones in which the termination of axon meets the surface of a single cells (simple synapsis) and such ones where the same axon is connected with two cells (complex synapsis).

Knowing the conditions found, there arises spontaneously the question, what the efferent synapses are good for, when nowadays it seems to be proved physiologically that the carotid bodies serve the chemoreception. Of course, it could also be thought that the old opinion is right according to which the nerve fibres of the glomus cells are effectors that regulate the secreting processes in the cells. In this sense the carotid bodies could not be considered as receptive organs. This opinion would prove to be right, founded on an objective interpretation of the conditions observed but in the case if the synapses of efferent type were seen in the electron microscopic pictures in a quantity corresponding to the number of terminal rings seen under the light microscope. But this is not the case. The truth is that in the pictures obtained in an electron microscope only a very low number of typically efferent synapses can be seen. Therefore, the opinion, mentioned above, appears to be incorrect and we can not help supposing that the efferent synapses are moving the vessel walls and are efferent bases of the reflex arch the sensory bases of which are given by the pressoreceptors mentioned above or are modulators of the glomic cells as it has been suggested by BISCOE and STEHBENS, (1966) too. As among these synapses there are some forms having oval synaptic vesicles and others having roundish vesicles, according to UCHISONO's theory (1965) we might also think that the former ones are inhibiting synapses (inhibitors) while the latter ones are excitatory in nature (excitators).

Summary

As a result of our investigations with electron microscope on human carotid bodies removed surgically owing to asthma bronchiale, we have establish the following:

Fig. 10. Homo: carotid body. Efferent synapses. Ax — axon, Pr — presynaptic membrane, S — synaptic space, Po — postsynaptic membrane, Cl — cluster, Pro — process, of chemoreceptor cell, M — mitochondrium. x 75 000.

1) The glomus cells that form the main mass of the carotid bodies can be classified into two groups, one of them are the chemoreceptor cells, the other the capsel cells.

2) The chemoreceptor cells are roundish, their cytoplasm is characterized by the well developed endoplasmic reticulum, the extensive GOLGI complexes, the large crista type mitochondria, the osmiophile bodies, the polymorphous lysosomes, cilia, microvilli and the wide desmosomes.

3) The capsel cells are elongated, their cytoplam is surrounds the likewise long homogenous nucleus in the form of a narrow border. The cytoplasmic inclusions are not developed the cells have some role in covering the nerve fibres.

4) The nerve fibres that mostly belong to the sensory system of the glossopharyngeal nerve reach the chemoreceptor cells covered in SCHWANN cells, resp. capsel cells and after having lost their cover, form synapses with them.

5) The majority of synapses belong to the afferent type. In these the synaptic vesicle clusters and membrane thickenings are lacking. These are the stimulus recipients of the chemoreceptor cells.

6) A little fraction of synapses displays the typical synapsis form. In all of them the characteristic components of the synapses are present. We consider these forms as efferent synapses.

7) As the number of efferent synapses is extremely low, the opinion seems to be correct according to which these are the moving bases of reflex arches, whose sensory bases are the pressoreceptors lying in the wall of blood vessels or modulators of the glomic cells.

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